EVIDENCE THAT DIMERS REMAINING IN PREINDUCED *ESCHERICHIA COLI* B/r Hcr$^+$ BECOME INSENSITIVE AFTER DNA REPLICATION TO THE EXTRACT FROM *MICROCOCCUS LUTEUS*

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ABSTRACT In *Escherichia coli* B/r Hcr$^+$ irradiated with two separate fluences, dimer excision is prematurely interrupted. The present study was designed to follow the fate of dimers remaining unexcised. The results imply that these dimers (or distortions containing dimers) are transformed on replication from the state of sensitivity to the state of insensitivity to endonuclease from *Micrococcus luteus*. This conclusion is based on the following findings: (*a*) dimers were radiochromatographically detectable in DNA replicated after UV, which indicated that they were tolerated on replication. (*b*) Similar amounts of dimers were detected radiochromatographically both in DNA remaining unreplicated and DNA twice replicated after UV. This, along with the low transfer of parental label into daughter DNA, indicated that dimers remained *in situ* in parental chains. (*c*) Immediately after UV, all parental DNA contained numerous sites sensitive to the extract from *M. luteus*. 2 h after UV, a portion of parental DNA still contained a number of endonuclease-sensitive (Es) sites, while another portion of parental DNA and all daughter DNA were free of Es sites. (*d*) The occurrence of parental DNA free of Es sites was not temporally correlated with dimer excision, but with the first round of DNA replication. (*e*) The amount of DNA free of Es sites corresponded to the amount of replicated DNA. (*f*) Separation of replicated and unreplicated DNA, and detection of Es sites in both portions separately showed that the replicated DNA was almost free of Es sites, whereas unreplicated DNA contained a number of such sites.

INTRODUCTION

The bulk of pyrimidine dimers produced by UV irradiation in DNA molecules of the wild type of *Escherichia coli* cells can be eliminated prereplicatively through excision repair (Setlow, 1967); a minor part is supposed to be tolerated postreplicatively by recombinational repair (Rupp and Howard-Flanders, 1968; Rupp et al., 1971).

In excision-deficient cells sites sensitive to T4 endonuclease V have been detected in DNA daughter chains synthesized on dimer-containing templates (Ganesan, 1974). Since T4 endonuclease V appears to be specific for dimers (Yasuda and Sekiguchi, 1970; Friedberg, 1972; Minton et al., 1975), the logical conclusion has been drawn that these sites may represent dimers transferred from parental into daughter strands through recombinational repair (Ganesan, 1974). It has been proposed that dimers remaining may be repaired through their dilution in such a way that about half of the original amount is transferred from parental into daughter strands during each replication cycle (Ganesan, 1975; Ganesan and Seawell, 1975).

Another type of postreplication repair suggests a synthesis of continuous DNA daughter strands on damaged templates (Radman, 1975; Witkin, 1975*a*, 1975*b*). This type of replication is supposed to be error prone, dependent on *recA* and *lex* loci, and related to UV-inducible functions (Devoret et al., 1975; Witkin, 1976), designated as SOS repair (Radman, 1974, 1975).

In an effort to account for phenomena that cannot be explained by the above-mentioned modes of repair, copy choice excision repair (Clark and Volkert, 1978), excision-dependent postreplication repair (Green et al., 1976) and incision-dependent copy choice bypass (Sedliaková et al., 1975*b*) have been postulated.

E. coli B/r Hcr$^+$ cells, unlike mammalian cells, perform efficient dimer excision when irradiated in the exponential phase of growth. If, however, before UV irradiation, they are exposed to thymine starvation (Sedliaková et al., 1974) and/or irradiated by a nonlethal UV predose (Sedliaková et al., 1978*a*), they behave like mammalian cells, leaving numerous dimers unexcised. The present study has been designed to elucidate the fate of dimers remaining in *E. coli* B/r Hcr$^+$ irradiated with inducing and lethal fluences.

MATERIALS AND METHODS

Bacterial Strain and Culture Conditions

A strain of *E. coli* B/r Hcr$^+$ thy$^-$ trp$^-$ was used. The glucose salt medium has been previously described (Sedliaková et al., 1966). Concentration of essential compounds added to the medium was 2 and 14 μg/ml of thymine and tryptophan, respectively. The medium with heavy isotopes for density labeling of DNA contained 0.1% $^{15}NH_4Cl$ (99% atom purity) instead of 0.2% $^{14}NH_4Cl$ and 0.1% [^{13}C]glucose (78.2% atom purity) instead of 1% [^{12}C]glucose. This medium was designated as heavy medium, and the culture medium of normal composition was referred to as light medium.

In all the experiments, cells were cultured in complete heavy or light medium up to a density of $\sim 2 \times 10^8$ cells/ml, then transferred to the medium lacking tryptophan. After 90 min starvation for essential amino acid, cells were resuspended in minimal medium and either irraditated or not with inducing fluence. Both parts were supplemented with essential compounds and cultured for 60 min. Then the cells were transferred to the minimal medium and irradiated with lethal fluence. After irradiation, the cells were cultured in the complete medium which contained 4 μg/ml thymidine instead of thymine.

Schedule of Experiments

IF + LF: Try$^-$ 90 min $\longrightarrow$ 30 J/m^2 $\longrightarrow$ Try$^+$ 60 min $\longrightarrow$ 50 J/m^2 $\longrightarrow$

LF: Try$^-$ 90 min $\longrightarrow$ No UV $\longrightarrow$ Try$^+$ 60 min $\longrightarrow$ 50 J/m^2 $\longrightarrow$

Ultraviolet Irradiation

Cells suspended in minimal medium (2–5 $\times$ 10^8 cells/ml) were irradiated with a Philips 15W TUV germicidal lamp emitting light predominantly of a wavelength of 254 nm (N. V. Philips Glocilampenfabrieken, Eindhoven, Netherlands). The incident-dose rate was 0.63 J/m^2 s. Depth of the irradiated layer was 1–2 mm; the suspension was manually stirred during irradiation. The UV-inducing fluence was 30 J/m^2; the UV lethal fluence was 50 J/m^2. Cultures were kept in the dark or in yellow light after irradiation to prevent photoreactivation.

Isolation of DNA and Isopycnic CsCl Centrifugation

In experiments in which the thymine-containing dimer:monomer ratio was followed, DNA was isolated by the lysozyme-pronase procedure described by Hanawalt and Cooper (1971). 0.5-ml samples of native DNA were added to 3.0 ml CsCl solution to reach a final density of 1.725 g/cm^3. Samples with CsCl solution were homogenized in glass homogenizers and transferred to polyallomer tubes for the 50Ti rotor of a Beckman L2-65B ultracentrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). Centrifugation was performed at 35,000 rpm. for 40 h at 20°C. After centrifugation individual fractions were collected on Whatman No. 3 paper strips (Whatman, Inc., Clifton, N.J.). The strips were washed in 5% trichloracetic acid and ethanol and radioactivity of the high molecular weight fraction was determined in a Packard Tri Carb 2450 scintillation counter (Packard Instrument Co., Downers Grove, Ill.). For determination of dimers in replicating and nonreplicating DNA, top fractions of heavy-heavy (HH) and heavy-light (HL) double-stranded DNA were collected. HL DNA was rebanded for determination of dimer content. Dimers were estimated in HH and HL DNA and H single strands derived from HH and HL double strands. (DNA was denatured after dialyzing by heating to 95°C for 15 min and then by fast chilling. 0.5-ml samples of denatured DNA were added to 3.0 ml CsCl solution to reach a final density of 1.740 g/cm^3). In separate experiments LL DNA was collected and rebanded for detection of parental label in DNA daughter chains.

In experiments in which endonuclease-sensitive (Es) sites were determined in replicating and nonreplicating DNA, cells were lysed by the Wilkins (1973) procedure. The lysates were either mixed with a CsCl solution without deproteinization of DNA (in vivo assay), or DNA was isolated by phenol extraction (in vitro assay) and then mixed with CsCl very gently to prevent DNA fragmentation. After centrifugation, the fractions of HH double strands were collected, dialyzed against NET buffer (0.01 M NaCl, 0.001 M EDTA, 0.001 M Tris, pH 8.0) and used for a determination of Es sites. HL regions were collected, rebanded by a second CsCl centrifugation, and treated as mentioned above.

Thymine Dimer Estimation

Fractions of DNA separated by CsCl density centrifugation were dialyzed and thymine-containing dimers were determined by two-dimensional paper radiochromatography (Carrier and Setlow, 1971*a*). For this purpose, the dialyzed fractions were dried and hydrolyzed with 0.2 ml of 98% HCOOH at 175°C for 45 min. Dimer and thymine spots were cut into 10-mm-wide strips and shaken for 30 min together with 5 ml of dioxan scintillation fluid and 0.3 ml water. Radioactivity was measured in a Packard Tri Carb 2450 scintillation counter.

Determination of Es Sites

Es sites were determined using the extract from *Micrococcus luteus*, which is believed to introduce nicks specifically into DNA containing pyrimidine dimers (Wilkins, 1973; Paterson et al., 1973). The extract was prepared by the method of Carrier and Setlow (1970), modified according to Carrier (personal communication). The method consists of four steps: (*a*) lysis of *M. luteus* cells and preparation of crude extract; (*b*) ammonium sulphate fractionation; (*c*) column chromatography on DEAE-cellulose; and (*d*) pooling of UV endonuclease active fraction by precipitation with ammonium sulphate.

Es sites assay described by Wilkins (1973) was performed as follows: 1-ml samples were harvested; washed once in 0.1 M Tris-HCl buffer, pH 7.6, 0.015 M NaN_3; and pelleted by centrifugation at 0°C. Cells were concentrated 10-fold in 0.1 M Tris-HCl, pH 7.6, containing 10% sucrose. In the in vivo assay, to each 40-μl sample of bacterial suspension 15 μl of lytic solution (equal volumes of 3.4 mg/ml lysozyme and 7.25 mM EDTA, both dissolved in Tris-HCl buffer, pH 7.6) and, after 10 min, 200 μl water were added. 50 μl of an incubation mixture, pH 8.0, containing 0.5 M Tris-HCl, 0.1 M β-mercaptoethanol, 0.01 M EDTA, and 3 μg of unirradiated calf thymus DNA was added and then incubated with 30 μl of UV-endonuclease for 30 min at 37°C.

In the in vitro assay, to each 250-μl sample of bacterial suspension 100 μl of lytic solution and, after 10 min, 1,000 μl water were added. Then sarkosyl was added to a final concentration of 0.5% and mixed. The suspension was deproteinized with redestilled phenol saturated with 0.1 M Tris-HCl buffer, pH 7.6.

After extracting with phenol, the aqueous phase was collected and dialyzed against NET buffer diluted 1:10. To a 100-μl sample of deproteinized lysate 20 μl of an incubation mixture (the composition of lytic and incubation solutions was the same as in the in vivo assay) was added and then incubated with 30 μl of UV-endonuclease for 30 min at 37°C. In both cases a control sample without enzymatic treatment was held in an ice bath. Except for incubation, all treatments were carried out at 0°C. Reaction was terminated by layering the suspension of about 2×10^6 cells onto 4.8-ml alkaline 5–20% sucrose gradient (in 0.1 M NaOH-0.5% sarkosyl) overlaid with 0.2 ml of 0.5 M NaOH-0.5% sarkosyl. Gradients were centrifuged at 35,000 rpm. at 20°C for 100 min in an SW 50.1 rotor in a Beckman L2-65B ultracentrifuge; fractions were collected from each gradient onto Whatman No. 3 paper strips. The strips were washed and prepared for counting as described by Carrier and Setlow (1971*b*). Radioactivity was determined as before.

DNA Molecular Weight Calculation

The weight average molecular weight (M_w) was calculated from the formula (Seeberg and Strike, 1976)

$$M_w = \frac{\Sigma Mi \cdot Ci}{\Sigma Ci},$$

where Mi represents molecular weight for each DNA fraction calculated from the formula $D_1/D_2 = (M_1/M_2)^k$ (Burgi and Hershey, 1963), with the molecular weights of T2 and T7 phage DNA used as standards. Single-strand molecular weight T2 DNA = 67×10^6; T7 DNA = 13.2×10^6 (Studier, 1965). D is a distance sedimented by a DNA molecule. Ci represents the amount of radioactivity in each fraction. The number of single-strand breaks per 10^8 daltons of irradiated DNA (N) was calculated from the formula (Paterson, 1978)

$$N = 10^8 \left[\frac{1}{M_n + E} - \frac{1}{M_n - E}\right], \qquad M_n = \frac{M_w}{2},$$

where $M_n \pm E$ are the number of average molecular weights after (+) and before (−) endonuclease treatment (E).

RESULTS

Dimers remaining unexcised because of a foregoing induction may be managed by cells in the following way: (*a*) They need not be subject to repair at all if they occur only in DNA remaining unreplicated. (*b*) In replicating DNA, they might either be gradually diluted through transfer into DNA daughter chains, or remain *in situ*, being tolerated through a recombination and/or a replicational bypass.

In our experiments dimers were determined separately both in DNA replicating after UV and in DNA remaining unreplicated. Data obtained indicate (compare columns I, II, and III in Fig. 1) that about half of the original amounts of dimers remain unexcised, being similarly distributed into DNA replicating and that remaining unreplicated. This finding is in agreement with previous observations (Meyn et al., 1974; Sedliaková et al., 1975*a*), and suggests that in culture with incomplete dimer excision, similar amounts of dimers occur in both DNA replicated and remaining unreplicated. Thus, under our conditions 1,000–2,000 dimers/cell were in some way managed on replication.

To determine whether dimers are repaired through their gradual dilution, the ratio of thymine-containing dimers to thymine monomers in nonreplicated and twice-replicated

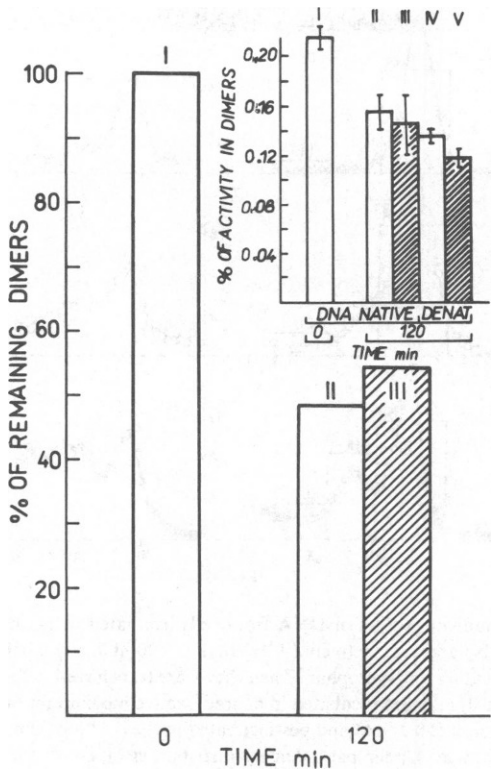

FIGURE 1 Thymine dimer content in both unreplicated (HH) and replicated (HL) DNA from cells irradiated with inducing and lethal fluence. For conditions see schedule of experiments and legend to Fig. 2*A*. Fractions of HH and HL DNA indicated in Fig. 2*A* (middle part and bottom) were pooled. HH and HL regions of the sample taken 120 min after UV (Fig. 2*A*, bottom) were rebanded by a repeated centrifugation. Dimers were estimated in I, unreplicated (HH), native DNA taken immediately after UV; II, unreplicated (HH), native DNA taken 120 min after UV; III, replicated (HL), native DNA taken 120 min after UV. *Inset:* I–III, the same but in nonrebanded DNA; IV, unreplicated (H), denatured DNA derived from HH, taken 120 min after UV; V, twice replicated (H), denatured DNA derived from HL, taken 120 min after UV. Activity in thymine spots was 500,000 cpm on average. The indicated values are averages from four experiments. Cell survival was 5.11×10^{-2}. Note that in these experiments inhibition of dimer excision was more pronounced than that shown in Fig. 5. This is due to different culture conditions.

DNA[1] and the amount of [^{3}H]thymine (a parental label) occurring in daughter DNA were determined. Our estimate was based on the following principles: if dimers were transferred into daughter DNA selectively (i.e., with a small amount of adjacent nucleotides), this should manifest itself by a marked decrease of the dimer:monomer ratio in parental DNA after each replication cycle. On the other hand, if dimers were transferred nonselectively (i.e., along with an amount of nucleotides so that the dimer:monomer ratio would remain unchanged) this

[1]In a separate experiment distribution of daughter label into HL hybrid and LL daughter region was determined 2 h after UV. The sum of counts per minute found in the LL region was twice that found in the HL region, indicating that parental DNA in the HL hybrid was, on the average, replicated twice. See the insert in Fig. 3, 120 min.

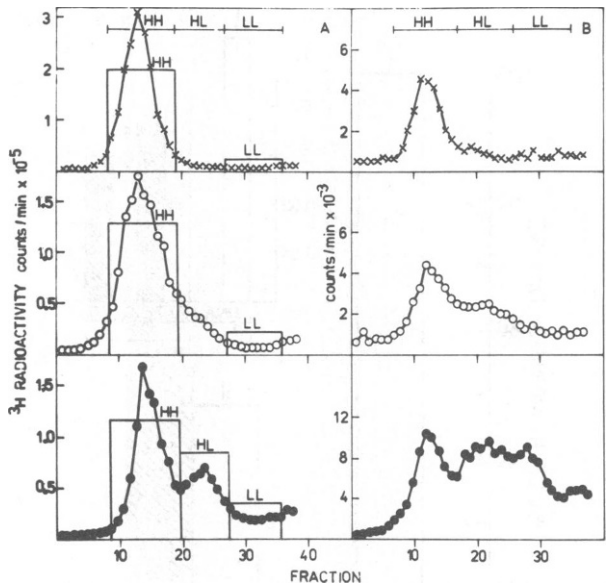

FIGURE 2 (*A*) CsCl gradient profiles of DNA from cells irradiated with inducing and lethal fluences. Cells were density ($^{13}C^{15}N$) and radioactively ([^{3}H] thymine 20 μCi/ml) labeled during the exponential phase of growth and starvation for tryptophan. Then they were transferred to the light medium, irradiated with inducing fluence (30 J/m^2) and incubated in nonradioactive medium for 60 min. The cells were then irradiated with lethal fluence (50 J/m^2) and postincubated in the cold medium, which contained 4 μg/ml thymidine instead of thymine. Upper part: density distribution of DNA from unirradiated cells (×); middle part: density distribution of DNA from cells irradiated with inducing and lethal fluences taken immediately after irradiation (O); lower part: density distribution of DNA from cells irradiated with inducing and lethal fluences and postincubated after lethal fluence for 120 min (●). LL regions indicated in the Figure were rebanded in CsCl gradients. (*B*) Density distributions of the rebanded DNA sedimented originally in LL region.

should manifest itself by the presence of large amounts of parental label in DNA daughter chains. Since neither a disappearance of dimers (in Fig. 1 compare columns II and III, and, in the inserts, columns II and III and columns IV and V) nor an occurrence of any significant amount of parental label in the daughter DNA (Fig. 2) were observed, the possibility that significant amounts of dimers remaining were repaired through gradual dilution of dimers could be excluded. Thus, it appears that the bulk of dimers remained *in situ*, and were not transferred.

To obtain more information about the mode of toleration of dimers on replication, the occurrence and elimination of sites sensitive to the endonuclease-containing extract from *M. luteus* (Es sites) were investigated. As evident from data shown in Fig. 3, Es sites gradually disappeared from a smaller part of parental DNA, while the greater part contained numerous Es sites during the whole period of observation. On the other hand, incubation with this extract did not decrease the molecular weight of newly synthesized DNA (Fig. 4). This indicated that daughter DNA was free of Es sites. The question arose as to what the mechanism causing elimination of Es sites from the smaller part of parental DNA was. Since the amount of DNA free of Es sites corresponded to the amount of replicated DNA (see Fig.

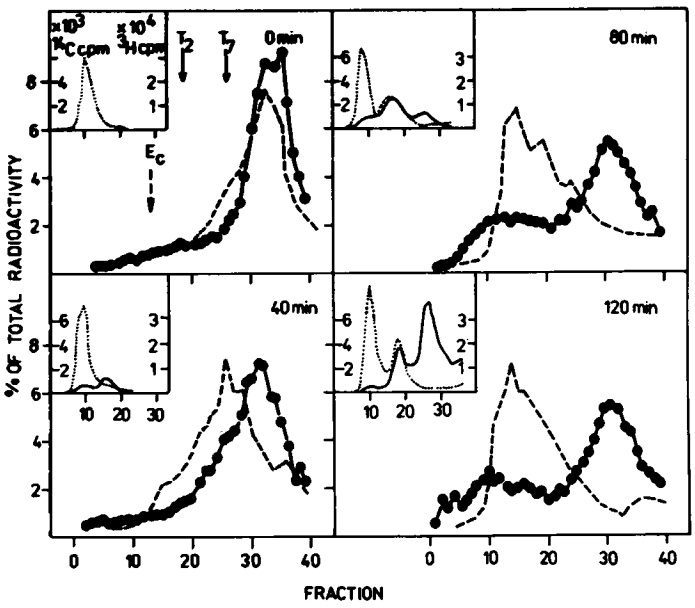

FIGURE 3

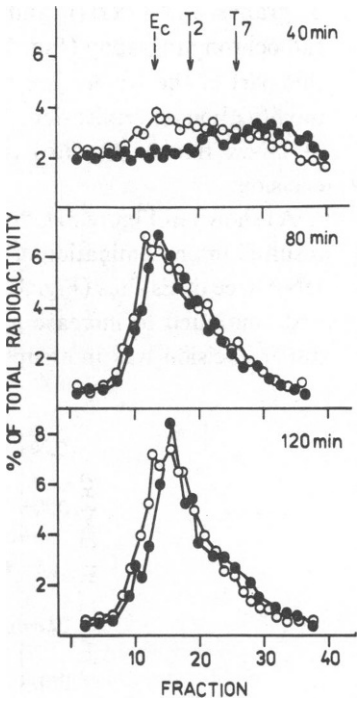

FIGURE 4

FIGURE 3 Es sites in parental DNA from cells irradiated with inducing and lethal fluences (●) or with lethal fluence only (–). During exponential phase of growth and starvation for tryptophan, cells were prelabeled with [^{14}C]thymine (1 μCi/ml). Then they were irradiated with inducing fluence (30 J/m^2) and incubated in nonradioactive medium for 60 min, or transferred to this medium and incubated for 60 min (noninduced cells). Both cultures were irradiated with lethal fluences (50 J/m^2) and postincubated in nonradioactive medium containing thymidine instead of thymine. Samples were taken at indicated time intervals after lethal fluence and treated with extract from *M. luteus* cells. Total counts per minute for each profile shown were:

LF:	0 min	2,735 cpm	IF+LF:	10,444 cpm
	40	2,393		7,200
	80	2,530		13,990
	120	3,243		5,622

Arrows indicate position of sedimentation of DNA from T2 and T7 phages and unirradiated *E coli* (E_c). CsCl gradient profiles of DNA from cells irradiated with inducing and lethal fluences are indicated in the insets. ---, heavy ^{14}C-labeled parental DNA; ———,light ^{3}H-labeled daughter DNA. Survival was 4.6×10^{-2} (IF+LF) and 2.7×10^{-2} (LF only).

FIGURE 4 Es sites in daughter DNA from cells irradiated with inducing and lethal fluences. Cells were cultured in nonradioactive medium during the exponential phase of growth and starvation for tryptophan. After irradiation with inducing fluence they were cultured in nonradioactive medium for 60 min and irradiated with lethal fluence. After irradiation with lethal fluence cells were postincubated in the complete medium in the presence of [^{3}H]thymidine (75 μCi/ml). Samples were taken at indicated time intervals after lethal fluence and treated (●) or not treated (○) with extract from *M. luteus* cells. Total counts per minute for each profile shown were: 40 min (untreated) 32,279 cpm; 80 min 150,254 cpm; 120 min 118,534 cpm; (treated) 12,803 cpm; 56,086 cpm; 51,010 cpm. Arrows indicate position of sedimentation of DNA from T2 and T7 phages and unirradiated *E. coli* (E_c).

3, graphs and inserts), and the replicated DNA contained dimers as detected by paper radiochromatography (Fig. 1), we have tentatively accepted the hypothesis that Es sites from this part of the DNA were not eliminated through dimer excision, but rather through their modification on replication. To verify this hypothesis we investigated whether the occurrence of DNA free of Es sites is temporally correlated with DNA replication or with dimer excision.

As shown in Figure 5*A*, dimer excision was most efficient during the 40 min after UV. This resulted in an elimination of some Es sites from the whole DNA, but not in the formation of DNA free of Es sites (Fig. 3). The amount of DNA free of Es sites increased 40 min after UV and continued to increase gradually until 70 min after UV (Fig. 5*B*). During that period dimer excision was interrupted (Fig. 5*A*) and the first replication cycle took place (Fig. 3,

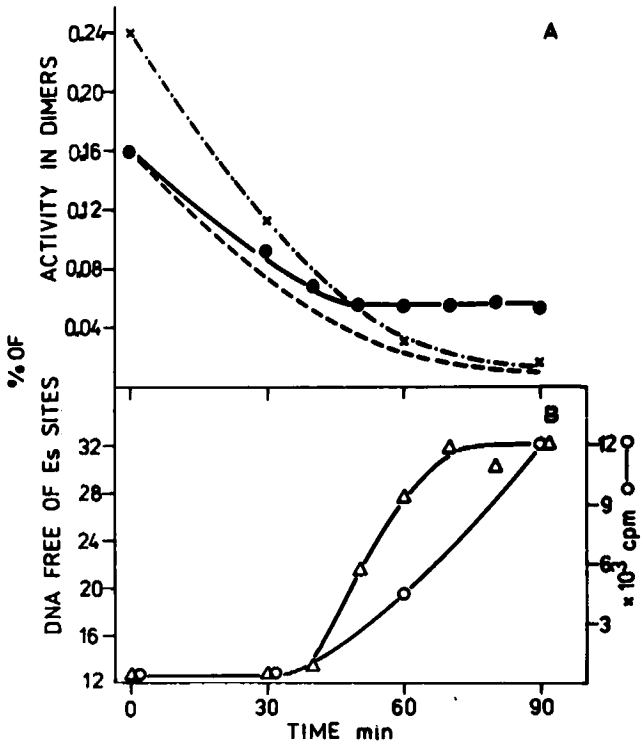

FIGURE 5 Time-courses of dimer excision, DNA synthesis and occurrence of DNA free of Es sites. (*A*) Excision of thymine dimers from cells irradiated with inducing and lethal fluences 30 + 50 J/m² (●), a single fluence 50 J/m² (--), or a single fluence 80 J/m² (×–). Cells were grown in complete medium with 0.5 μCi/ml [^{14}C]thymine up to amino acid starvation and then treated as indicated in Fig. 2*A*. Samples were withdrawn and dimers were estimated during postincubation after lethal fluence. Cell survival was 3.4×10^{-2} (30 + 50 J/m²) and 2.5×10^{-2} (50 J/m²). (*B*) DNA synthesis and percentage of parental DNA free of Es sites from cells irradiated with inducing and lethal fluences (30 + 50 J/m²). O, DNA synthesis. Cells were cultured as indicated in legend to Fig. 4. After lethal fluence the culture medium contained 1 μCi, 5.6 μg/ml of [^{14}C]thymidine. At indicated time intervals samples were taken and radioactivity of the high molecular weight fraction was determined. Δ, Percentage of parental DNA free of Es sites was calculated from profiles of parental DNA treated with extract from *M. luteus* cells in high molecular weight regions of the gradient. The percentage was calculated from similar profiles as indicated in Fig. 3.

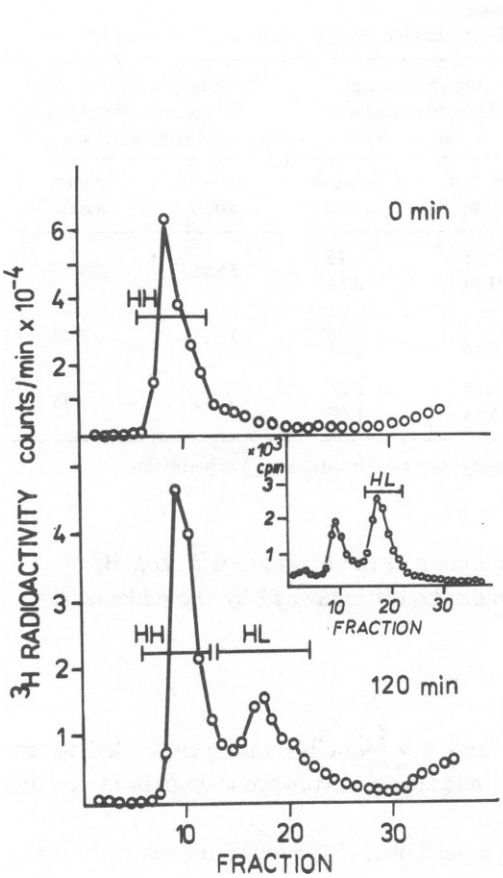

FIGURE 6 *A*

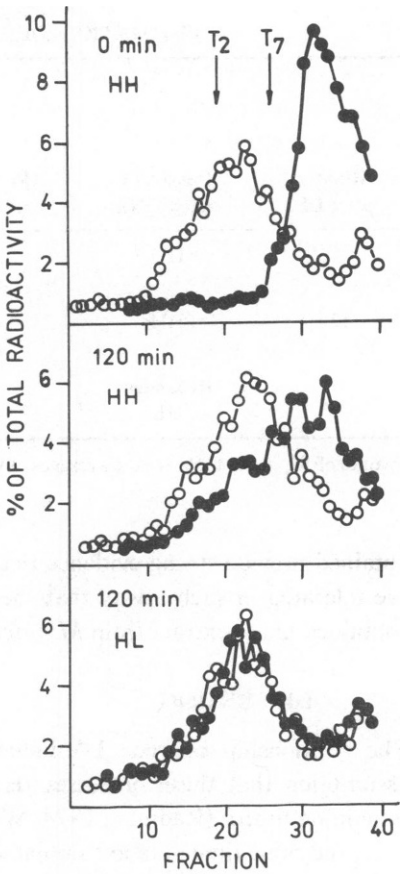

FIGURE 6 *B*

FIGURE 6 (*A*) CsCl gradient profiles of DNA from cells irradiated with inducing (IF) and lethal (LF) fluences. Cells were cultured and labeled as indicated in Fig. 2. Immediately after lethal fluence and 120 min after lethal fluence cells were lysed and DNA was separated by CsCl centrifugation. Fractions of HH and HL DNA indicated in the Figure were pooled and HL DNA was rebanded as illustrated in the insert. (*B*) Estimation of UV Es sites by in vivo enzymatic assay in both unreplicated (HH) and replicated (HL) DNA from cells irradiated with inducing and lethal fluences. Fractions of HH and rebanded HL double strands indicated in Fig. 6*A* were treated (●) or not treated (○) with extract from *M. luteus* cells. Total counts per minute for each profile shown were: 0 min HH (untreated) 9,296 cpm; 120 HH 3,867 cpm; 120 HL 3,215 cpm; (treated) 6,903 cpm; 3,812; 2,728.

insets). Thus, occurrence of DNA free of Es sites was not temporally correlated with dimer excision, but rather with DNA replication (Fig. 5).

To make sure that the DNA free of Es sites actually corresponded to the DNA replicated after UV, the replicated DNA was separated from the DNA remaining unreplicated by a repeated density gradient centrifugation (Fig. 6*A*). Es sites were detected separately in replicated and unreplicated DNA by both in vivo and in vitro assay (Paterson, 1978). As documented in Fig. 6*B* and Table I, the unreplicated DNA contained a significant amount of Es sites 2 h after UV, whereas the replicated DNA was almost free of Es sites. Thus, the data

TABLE I
ESTIMATION OF UV-Es SITES BY ENZYMATIC ASSAY

Minutes after LF	Density of tested DNA	UV-endo added	Weight-average molecular weight* ($M_w \times 10^7$) In vivo assay	Weight-average molecular weight* ($M_w \times 10^7$) In vitro assay	Number of single strand breaks per 10^8 daltons of irradiated DNA In vivo assay	Number of single strand breaks per 10^8 daltons of irradiated DNA In vitro assay
0	HH	–	5.38	1.45	56.79	103.85
		+	0.34	0.17		
120	HH	–	4.30	1.25	10.21	32.78
		+	1.35	0.41		
	Rebanded HL	–	4.16	1.20	0.86	1.85
		+	3.53	1.09		

*Values of M_w in the table are the averages of three (in vivo assay) and two (in vitro assay) experiments.

obtained provide strong evidence that dimers remaining in the preinduced *E. coli* B/r Hcr$^+$ are tolerated in such a way that they can no longer be distinguished by the endonucleases contained in the extract from *M. luteus*.

DISCUSSION

The relationship between UV-inducible repair and UV-inducible mutagenesis led to an assumption that these processes reflect an uvr-independent error-prone bypass of lesions remaining *in situ* (Radman, 1974; Witkin, 1975*b*).

At the same time data accumulated indicating a need of excision repair for some products, which proved to be inducible (Cooper and Hanawalt, 1972; Youngs and Smith, 1973; Cooper and Hunt, 1979). Rather inconsistent with this, however, excision repair was found to be inhibited by a foregoing induction. This has been observed with Weigle reactivation of phage λ (Boyle and Setlow, 1970), as well as with Chinese hamster cells exposed separately to *N*-acetoxy-2-acetylaminofluorene and UV (Ahmed and Setlow, 1977); this has also been observed in wild type bacterial cells namely, *E. coli* B/r Hcr$^+$, *E. coli* K-12 AB 2497, and *E. coli* 15 555-7 preinduced by thymine starvation (Sedliaková et al., 1974) as well as in *E. coli* B/r Hcr$^+$ (Sedliaková et al., 1978*a*), *E. coli* WP_2, and *E. coli* 15 555-7 preinduced by UV preinduction but not in UV-preinduced *E. coli* K-12 AB 2497 (Sedliaková et al., 1980*a*) or in thymineless preincubated *M. radiodurans* (Budayová and Sedliaková, 1977).

The data presented in this paper provide evidence that in wild type cells a UV-inducible repair pathway operates that enables tolerating lesions to remain unexcised. This process differs from toleration of lesions occurring in excision-deficient cells in several respects: (*a*) It is roughly as efficient as excision repair (see survival data in legends to Figs. 3 and 5), (*b*) it is uvr dependent, (*c*) it modifies lesions in such a way that they become insensitive to the endonucleases from *M. luteus* which could originally distinguish them, and (*d*) data obtained in our laboratory indicate that it is less error prone (manuscript in preparation).

The process in question is a complex one. Previous data (Sedliaková et al., 1978*b*) appear to indicate that it requires at least four genes to function, namely, *uvrA*, *uvrB*, *lexA* and *recA*.

From the fact that in this process, the *uvr* system is required for a step other than that of removal of dimers, it may be deduced that the process involves either a *uvr*-dependent replicational bypass of dimers (a copy choice mechanism) or a *uvr*-dependent gap-filling step through recombination or resynthesis. In either case the contribution of an intact homological duplex should be necessary to supply the damaged molecule with correct information. The mode in which sister duplexes may interact if DNA replication in one of them is interrupted was for a long time unknown. Recent findings about *recA*-dependent annealing of complementary strands from homological duplexes (Weinstock et al., 1979), and formation of *recA*-dependent D-loops and joint molecules (Cunningham et al., 1979) threw some light on this point. A recently reported process, designated as "cutting in trans" (a *uvr*-, *recA*-dependent single-strand incision introduced into a homologous duplex, Ross and Howard-Flanders, 1977; Cassuto et al., 1978) suggests interaction of *uvrA*, *uvrB*, and *recA* genes in a process different from excision. All these findings may be interpreted in favor of recombination as well as replicational bypass. However, the *recA*-dependent stable DNA replication (for the fact that in damaged cells several replication cycles can take place in the absence of initiating proteins, cf. Kogoma and Lark, 1975; Kogoma et al., 1979) indicates that *recA*-dependent processes cannot be reduced to filling some overlapping gaps. It rather indicates that in damaged cells a different mode of replication is triggered.

The fact that the endonucleases from *M. luteus* do not distinguish dimers in replicated DNA suggests that they distinguish local distortions of DNA molecules associated with dimers, rather than dimers themselves. This is consistent with recent findings (Riazuddin and Grossman, 1977). Since dimers are not distinguished even in extracted DNA, the loss of Es sites cannot be ascribed to binding proteins which might cover dimers and make them insensitive to the action of the enzyme. A suitable explanation may be that the denatured regions containing dimers are reduced on replication, which manifests itself as disappearance of Es sites. It appears that excision-proficient cells can normalize these distortions on replication, leaving dimers *in situ*, whereas excision-deficient cells cannot do so (Sedliaková et al., 1980*b*).

Some time ago it was shown that the *recA* mutation sensitizes cells to UV radiation more than a *uvr* mutation (Howard-Flanders and Boyce, 1966). More recently it has been shown that excision-proficient cells can tolerate greater amounts of unexcised dimers than can excision-deficient cells (Sedliaková et al., 1975*b*; 1977). In conformity with this it has been found that *uvrA* and *uvrB* products may be involved in postreplication repair (Ganesan and Seawell, 1975; Rothman and Clark, 1977). All these phenomena were hardly understandable from the view of the original excision-recombinational repair conception. They are, however, becoming clear in the light of new findings indicating that *uvrA*, *uvrB*, and *recA* functions are involved in some replicational (and/or postreplicational) steps taking advantage of both homologous duplexes.

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